Analysis of lens redox status in cataracts from rat models of type 1 and 2 diabetes

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ABSTRACT

Purpose. To identify differential changes in redox status underlying type 1 (T1DC) and type 2 (T2DC) diabetic cataract (DC) formation in rat. Material and methods. Rat models of type 1 and 2 diabetes were respectively induced, and cataract progression was examined weekly. At week eight the levels of reduced glutathione (GSH), oxidized glutathione (GSSG), nicotinamide adenine dinucleotide 2'-phosphate reduced tetrasodium salt (NADPH), and glucose-6-phosphate dehydrogenase (G6PD) activity were measured and compared with those in controls.

Results. Cataracts were observed two more weeks earlier in type 1 than type 2 diabetic rats. Furthermore, cataracts were progressed more slowly in T2DC compared with those in T1DC. Lens GSH levels were decreased 4-fold and 2-fold in type 1 and type 2 diabetic rats, respectively, compared with levels in normal control rats. NADPH levels were showed 2.4-fold and 1.5-fold decreases in type 1 and 2 diabetic rats, respectively, compared with normal controls. Similar changes were found in the activity of G6PD.

Conclusions. The results suggest that GSH and NADPH loss might be important pathological mechanisms in T1DC and T2DC formation. The «fast» progression of cataracts in type 1 diabetic rats might be due to the more severe oxidative stress than in type 2 diabetic rats.

Key words: cataract, model in rat, type 1 and type 2 diabetes mellitus, redox oxidative status.

No author has a financial or proprietary interest in any material or method mentioned.

Cataract represents one of the most frequent eye complications in type 1 and type 2 diabetes mellitus. A type 1 diabetic cataract (T1DC) is characterized by bilateral snowflake-type cortical deposits and/or a subcapsular cataract [2]. These opacities often evolve rapidly. The type 2 diabetic cataracts (T2DCs) are morphologically similar to senile cataracts, and generally progress much more slowly than in T1DCs. Many studies have focused on the T1DC. However, slowly progressive, T2DC, has been researched in less detail. Considering the slow process of type 2 DCs, studies on such cataractogenesis might reveal novel mechanisms underlying DCs. In the present study, we used this type-2 diabetic model, together with the typical type 1 diabetic model in rats, to investigate the common and respective redox status changes in the two types of DC lenses with comparisons to controls, by metabolism measurements.

**MATERIAL AND METHODS**

A total of 60 age-matched male Wistar rats (7 weeks of age) were divided into 4 groups: the group N (normal control) rats were fed a standard diet (12% of calories as fat); in the group T1DC rats, type 1 diabetes was induced by an streptozotocin (STZ, 60 mg/kg) injection following a 4-week standard diet, after which the standard diet was continued; the group T2DC rats were administered a relatively moderate amount of STZ (50 mg/kg) following a 4-week high-fat diet (HFD, 40% of calories as fat), after which the HFD was continued, as described previously [6, 16]; and the group H rats (the HFD control) were fed the HFD constantly, without diabetes induction. Blood glucose and insulin levels were determined weekly, and homeostasis model assessment-insulin resistance (HOMA-IR) was used to evaluate insulin resistance (HOMA-IR = fasting blood glucose level \times fasting insulin level \div 22.5). The group T1DC rats with non-fasting blood glucose levels > 13.8 mM were considered to have type 1 diabetes, and the group T2DC rats with non-fasting blood glucose levels > 13.8 mM and significantly (P<0.01) increased HOMA-IR values were considered to have type 2 diabetes. The induction of the type 1 and type 2 diabetes was confirmed 3 days after STZ injection. Cataract progression was examined weekly. Based on opacity analysis, characteristic cataracts could be observed at week 8 after the induction of diabetes in the two types of diabetic rats. Therefore, at this time point, lenses were extracted from each group and frozen in liquid nitrogen for subsequent biochemical analyses.

Lenses from different individuals (n=5) from each group were weighed and deproteinized by homogenization in perchloric acid. The levels of reduced glutathione (GSH), oxidized glutathione (GSSG), were measured in concentrated K$_2$CO$_3$-neutralized perchloric acid extracts using a spectrophotometer and enzymatic procedures, as described previously [9, 11, 12]. For the estimation of NADPH, the lenses (n=5/group) were weighed and homogenized in 0.2M KOH. Next, an equal volume of 0.23M KH$_2$PO$_4$ was added for neutralization, and the mixtures were centrifuged. The NADPH levels were measured in the supernatant following the method of Giblin and Reddy [4]. Five lenses from each group were used for glucose-6-phosphate dehydrogenase (G6PD) activity analysis. Each lens was homogenized in 50mM PBS (pH 7.0) and subsequently centrifuged. The supernatant was then collected. The reaction mixture contained 3.3mM MgCl$_2$, 55mM Tris buffer (pH 7.8), 100mM glucose-6-phosphate, and 6mM NADP. The G6PD activity was measured as previously described [1].

Statistical analysis was performed by Student's t-test and one-way ANOVA using SPSS version 17.0 software (SPSS Inc., Chicago, IL). P<0.05 was considered to represent a significant difference.

**RESULTS**

Before STZ injection, the rats given the HFD (groups T2DC and H) had significantly higher HOMA-IR values compared with the rats fed the standard diet (P<0.01). The prediabetic state is characterized by insulin resistance (IR), which revealed the natural history of type 2 diabetes. After induction of diabetes, the non-fasting blood glucose concentrations in the type 1 and type 2 diabetic groups both increased (~3-fold) significantly (P<0.01), and the blood glucose levels in the two groups were similar until week eight. Cataracts were observed in type 1 diabetic rats after the 3-week duration of diabetes on average, whereas in type 2 diabetic rats, cataracts appeared after two more weeks. Cataracts in type 2 diabetic rats progressed much more slowly than those in type 1 diabetic rats.

To investigate antioxidant status in DC and control lenses, the levels of GSH and GSSG in lenses were measured. Lens GSH levels in type 1 diabetic rats were decreased 4.0-fold compared with levels in normal control rats, whereas GSSG levels were similar between the two groups (figure 1). In type 2 DC lenses, GSH levels were decreased 2.0-fold, whereas GSSG levels were increased (P<0.05) compared with levels in normal controls. As a result, the lens GSH/GSSG ratio was lower in type 1 and type 2 DC lenses compared with normal controls, and GSH and GSSG levels and the GSH/GSSG ratio were higher in type 2 DC lenses compared with type 1 DC lenses. There were no significant differences in the three parameters between normal and HFD controls. Notably, the lower levels of GSH in the type 1 DC lenses revealed more severe oxidative damage.

The changes in glutathione levels could be partially related to the hexose monophosphate shunt (HMPS), which produces NADPH to regenerate GSH from GSSG. Lens NADPH levels were measured and showed 2.5-fold and 1.5-fold decreases in type 1 and 2 diabetic rats, respectively, compared with normal controls (figure 2). Moreover, similar changes were found in the activity of G6PD, which is the
rate-limiting enzyme of the HMPS that catalyzes the first step in NADPH production.

**DISCUSSION**

Oxidative stress occurs in the lens early during the course of diabetes and is manifested by depletion of the main biological antioxidant, GSH, and an increase in the GSSG/GSH ratio [10, 14]. The type 1 diabetes-induced changes in glutathione levels in this study are consistent with previous studies [7, 8]. The significant decrease in GSH levels is not solely due to utilization of glutathione [5, 13]; another factor causing decreased GSH levels might be decreased GSH synthesis [3, 15]. Furthermore, Lou et al [7] observed that the GSH loss is the result of the lens’ impaired ability to concentrate the amino acids required for GSH biosynthesis, coupled with faster GSH efflux under hyperosmotic conditions. The reduction in GSH levels and the concomitant increase in GSSG levels in type 2 DC lenses might have been mainly due to the increased utilization of GSH. The lower levels of GSH in type 1 DC lenses compared with type 2 DC lenses revealed more severe oxidative damage in the type 1 diabetic lenses, which might contribute to faster cataract progression. In addition, the accumulation of GSSG relative to GSH in type 1 and 2 diabetic rats could also be related to the inability of lens cells to produce sufficient NADPH via the HMPS, which was supported by the decreased levels of lens NADPH and G6PD activities observed in the current study (figure 2).

**REFERENCES**


Received 15.10.2015